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TITLE: Noninvasive Detection of Lactate as a Biomarker of Response Using Spectral-Selective Multiple Quantum Editing Sequence
(SS-SeIMQC)

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14. ABSTRACT This application focuses on enhancing cancer care by developing non-invasive techniques to determine better biomarkers to improve diagnostic specificity and decrease the number of negative biopsies, and also as markers of response with novel targeted agents such as Trastuzumab and Bevacizumab. Last year, we have constructed radiofrequency coils suitable for studying orthotopic breast tumors, optimized SelMQC and SS-SelMQC sequences for qualitative lactate detection in phantoms. In this year, we made substantial progress in terms of implementing these sequences at 4.7T and two dimensional MR spectroscopic imaging of lactate using <i>in vivo</i> breast tumors. We also developed methods for T ₁ and T ₂ relaxation measurements to facilitate the quantification. We quantified lactate concentrations from whole tumor as well as localized lactate signal from 5mm slice. To understand the tumor heterogeneity, an important step towards understanding treatment response, we succeeded in the process of quantifying the lactate from each voxel of 2D CSI with phantoms and presently we are working on quantifying lactate in 2D CSI with all collected <i>in vivo</i> data.					
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Introduction

This application focuses on using lactate as a marker in breast cancer. Until now, choline has been used as a marker with 90% specificity. We propose to use lactate (Lac) as a surrogate marker of breast cancer using SS-SelMQC (1) using binomial spectral selective pulses as well as SelMQC (2) method. Lac detection is technically challenging as breast tissue has very high levels of lipid (Lip) and water. The main objective of the proposal is developing and evaluating a more effective method of detecting Lac non-invasively by magnetic resonance spectroscopy (MRS) techniques. Lac is present in very small quantities (milli moles) compared to Lip (moles) and water and the signal of Lac occurs in the same position as Lip, thereby making it very difficult to see. Treatment of breast cancers with novel targeted agents such as Trastuzumab and Bevacizumab have led to significant gains, although the drugs can be toxic. Breast tumors are usually sensitive to many drugs but subsequently develop resistance. There is strong interest in applying drugs that interfere with angiogenesis and signaling pathways related to breast cancer growth and metastasis. Low extracellular pH and high Lac levels were shown to be indicators of metastatic risk in breast cancer xenografts. Elevated Lac in biopsy samples was shown to correlate with increased risk of metastasis and poor patient survival in different aggressive cancers, while a decrease Lac levels observed in tumor response to radiation and chemotherapy. Therefore, non-invasive measurement of Lac may be an additional characteristic marker for breast cancer; it may improve diagnostic specificity, serve as an early marker of tumor response, and provide functional information about prognosis.

Body

Over the last year, we have made significant progress in acquiring the *in vivo* data with using breast tumors in mice proposed since in the previous year we had done most of the technical

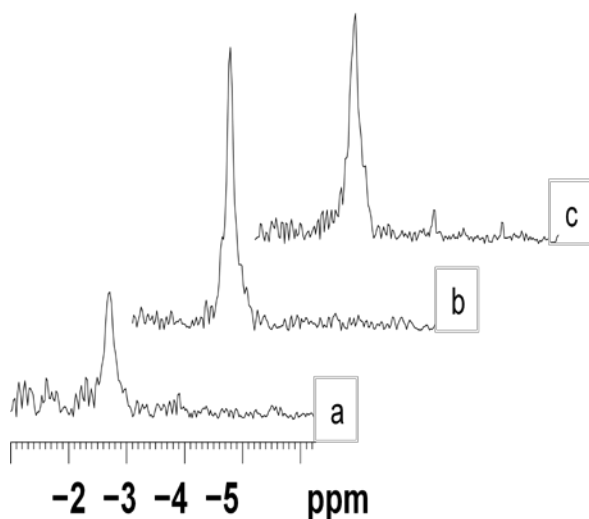


Figure 1: *In vivo* Lac spectra of MCF-7 tumor from 5 mm slice as a function of increasing tumor volume (a) 150 mm³, (b) 330 mm³ and (c) 750 mm³; TR=3s; Number of scans=16 and acquisition time= 48 minutes.

work necessary for Lac detection. In this time period of the proposal, we optimized RF coils suitable for orthotopic breast tumors at different tumor volumes. We also implemented and evaluated the sequence (1) for Lac detection in non-localized whole tumor spectra, one dimensional slice, and two dimensional (2D) chemical shift image (CSI), explored higher order binomial pulses for better Lip suppression, developed sequences for T₁ and T₂ measurements of lactate using SS-SelMQC for Lac quantification from 1D and 2D data as a function of increasing tumor volumes (**Figure 1**).

Quantitation: Absolute concentrations of Lac metabolite from MR data using multiple quantum editing can be calculated using two methods. These include the external reference quantification method,

which uses an external standard with the known concentration and internal reference method which uses water as a reference. We have used the external standard or substitution method and this method requires careful measurements of the relative power each time one acquires data and comparing the power required for different pulses when the subject (mice) is in place, vs that required when a phantom (known concentration) is in place. Recently, we are also exploring usage of water signal as reference for Lac quantification and compare with the external reference method.

As part of our research design, we want to determine if Lac is a marker of sensitivity to novel targeted drugs used in breast cancer xenograft models and to test if lactate concentrations may predict tumor aggressiveness using metastatic and non-metastatic breast cancer models. We implanted MCF-7, BT-474 and MDA-MB-231 tumors to test *in vivo* and tumor growth were measured (**Figure 2**). These were used as controls for Lac study proposed in our research design. We have also implanted few mice with SKBR-3 and MDA-MB-435 cell lines. Lac

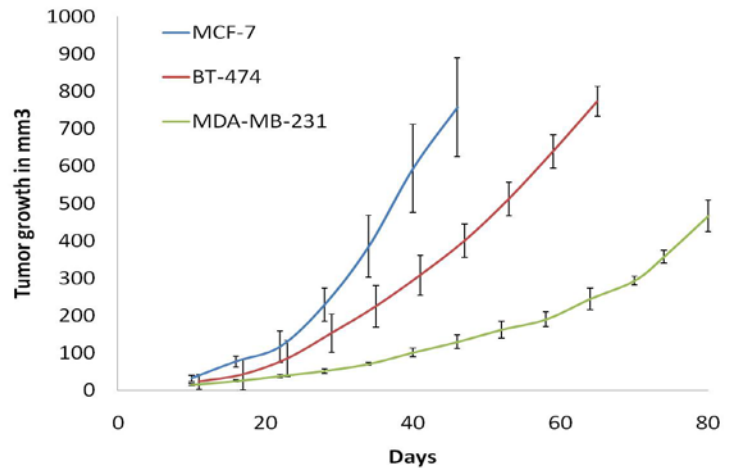


Figure 2: Plot of tumor volume of MCF-7, BT-474 and MDA-MB-231 cell lines induced breast tumors in mice. Doubling time for MCF-7, BT-474 and MDA-MB 231 cell lines are 8, 12 and 16 days respectively.

spectroscopic studies of MCF-7(0-800 mm³), MDA-MB-231(0-500 mm³), BT-474 (0-800 mm³) and analyzed the data. Now MDA-435 tumors are growing for control studies and MCF-7 and MDA-MB-231 tumors are growing for studying treatment Herceptin and Avastin respectively. SKBR3 tumors were able to grow only up to less than 100 mm³ and got necrotic. Although we could detect Lac from whole tumors, no detectable signal in 2D CSI data was observed. We will explore further on these tumors during this year.

Data acquisition: Six week old athymic nu/nu female mice were used for the study. For MCF-7, SKBR-3 and BT-474 mice, two days before the cell inoculation estrogen pellet was induced in the body. Five million cells were inoculated on the right inguino-abdominal region of the mammary fat pad of the mice and tumor growth was monitored every three days after one week of inoculation.

For MR studies, tumor bearing mice were anesthetized with isoflurane (1.0-2.5%) and compressed air. MRS lactate determination studies were conducted for tumors in the range of 100-800 mm³. Tumor bearing mice were typically studied 3-4 times.

One dimensional non-localized Lac MR spectra from whole phantom and 5 mm localized sagittal slice were collected using reported SS-SelMQC (1) using 11 binomial pulses for selective pulse excitation and inversion, and SS-SelMQC using 1331 binomial pulses. We also acquired T₁ and T₂ data from phantoms and *in vivo* breast tumors (**Table 1**) using newly

developed SS-SelMQC T_1 - and T_2 -variants similar to (3), for quantification of Lac concentrations.

To measure T_1 and T_2 using these newly developed sequences as part of the Lac quantification procedure, phantom studies were carried out in three different chemical environment conditions and with three different Lac concentrations. All the phantom preparation was carried out in physiological saline. The three different chemical environmental conditions include only lactate in saline, Lac in presence of lipid (Crisco fat) and in presence of contrast agent Gd-DTPA. The three different Lac solutions include 5mM, 15mM and 30 mM concentration. These three

different chemical environment and different lactate concentration was chosen to mimic the *in vivo* condition.

The tumor was placed inside a 2 turn home built 10 (upto 300 mm³) and 15 mm (> 300 mm³) diameter tuned coil.

MR spectroscopy

acquisition parameters:

11.25°, 22.5°, 45°, 33.75°, 67.5° and 90° pulse flip

angle three-lobe sinc

shaped pulses with 200 μ s

and 400 μ s pulse duration, a

pulse repetition of 3 s and

spectral width of 12.5 ppm.

Transmitter is set at CH

frequency of Lac and all other

experimental parameters are

chosen from ref (1). The ZQ

→DQ coherence transfer

pathway is selected with the

Gsel gradients in a ratio of 0:-

1:2; All 2D sets were collected

using 2D SS-SelMQC with 1st

pulse keeping to 90 slice

selection pulse. The pulse sequence parameters for the lactate editing experiments included 512

data points, 8 averages, TR=2 s and a spectral width of 2500Hz. A matrix size of 16×16, FOV of

20 mm (1.25 x 1.25 mm in plane resolution) was used. Two-dimensional chemical shift imaging

lactate maps were generated by selecting a 5 mm slice using a 1ms three-lobe Sinc pulse. The

2D CSI lactate map was visually co-registered with T_2 -weighted images of 5 mm slice thickness

from the center of the tumor. For lactate quantification, we calculated T_1 and T_2 correction

factors using T_1 -SS-SelMQC and T_2 - SS-SelMQC respectively. (These results were accepted for

presentation at ISMRM 2011 and in the process of submitting as a paper; Included in appendices).

Table1: ER PR and HER2 status with T_1 and T_2 of *in vivo* and phantom studies.

		ER/PR/HER2	T1 sec	T2 sec
In vivo	No of Mice			
MCF-7	6	+ + -	1.80±0.23	0.16±0.02
BT-474	6	+ + +	1.66±0.26	0.17±0.02
MDA-MB-231	3	- - -	1.37±0.07	0.12±0.00
In-vitro	Trials			
15	3		1.24±0.08	0.42±0.03
15 with lipid	3		1.20±0.04	0.42±0.02
15 with 25 μ M	3		0.66±0.03	0.36±0.03

T_1 -SS-SelMQC

180° (nonselective) – TI g_c – [45°_x – Δ_1 – 45°_x] (Lac CH₃ lipid) – τ – [45°_x – Δ_1 – 45°_x] (CH water) – t1/2, g_1 – [90°_x – Δ_2 – 90°_x] (CH₃ lipid) – t1/2, g_2 – [45°_x – Δ_1 – 45°_x] (CH H₂O) – g_3 , $\tau \pm t_1$ – acq

T_2 -SS-SelMQC

[45°_x – Δ_1 – 45°_x] (Lac CH₃ lipid) – TE'/2 – 180° (selective) – TE'/2 – [45°_x – Δ_1 – 45°_x] (CH water) – t1/2, g_1 – [90°_x – Δ_2 – 90°_x] (CH₃ lipid) – t1/2, g_2 – [45°_x – Δ_1 – 45°_x] (CH H₂O) – g_3 , $\tau \pm t_1$ – acq

Non-localized spectra were obtained with 16 transients for T_1 and 32 transients for T_2 measurements. In the T_1 -SS-SelMQC, T_1 measurement of Lac was performed with insertion of inversion ‘mao4’ shaped pulse with 2000 μ s pulse width, and varied the inversion recovery delay (0.1s to 1s) with 0.2 s increment and (1s to 10s) in steps of 2s before applying the SS-SelMQC with relaxation delay TR 10s. In T_2 -SS-SelMQC, Lac T_2 relaxation was measured by incorporating CH_3 selective 15 ms single lobe ‘sinc’ pulse, during the MQ-preparation period of SS-SelMQC. This allows inserting a variable delay time TE to measure Lac T_2 decay. In phantom study the echo time TE was varied from 0.02s to 1.2 s with 0.06 s increment. In in-vivo-study echo time TE was varied from 0.02s to 0.4s with 0.02 s increment. The T_1 measurement was done by subtraction method (4).

Data Processing:

T_1 and T_2 measurements: Nonlocalized and 1D slice-localized Lac spectral data were processed using Bruker X-win nmr software and lactate signals were integrated using the area under the peak. The integral values were used for the T_1 and T_2 calculation. The T_1 was calculated using $S = S_0(1 - 2\exp(-TI/T_1)) - S_0$ Where S is Signal intensity at variable inversion delay, TI is inversion delay and T_1 is spin-lattice relaxation time. The T_2 -relaxation delay was calculated using $S = S_0 \exp(-TR/TE)$, where TR is recycle delay and TE is Echo time. The Matlab was used to fit the curve.

2D CSI processing: Spatial Fourier transform and superimposition of the spectral grid on the corresponding T_2 -weighted image were performed using the 3DiCSI software package. The voxels within the tumor were then identified and the free induction decay (FID) from each tumor voxel was extracted as numerical text data. Preprocessing parameter apodization (0.2Hz), phase correction was done. The data for each FID was input to the JMRUI software package and the Lac resonance was fit in the time domain using AMARES (Advanced Method for Accurate, Robust, and Efficient Spectral fitting). The reference phantom 2DCSI set was processed similarly. For quantitation purposes, each tumor voxel was referenced to a phantom voxel at the exact same location. The tumor voxel Lac peak area, the reference voxel Lac peak area, and the appropriate T_1 and T_2 factors for tumor and phantom studies and were then used to quantify the tumor voxel Lac using following equation.

$$C = C_{ref} f_{CL} \frac{A}{A_{ref}} \frac{(1 - \exp(-TR/T_{1ref}))}{(1 - \exp(-TR/T_1))} \exp\left(-TE\left[\frac{1}{T_{2ref}} - \frac{1}{T_2}\right]\right).$$

Where , C_{ref} is concentration of Lac in the phantom with known concentration, f_{CL} is coil loading factor for phantom and tumor . A & A_{ref} are area of Lac signal in *in vivo* and standard phantom respectively. T_1 & T_{1ref} are spin-lattice relaxation time of Lac in *in vivo* tumor and in standard phantom respectively. T_2 and T_{2ref} are spin-spin relaxation time of Lac in *in vivo* tumor and in standard phantom respectively.

Results: Lac spectroscopic studies of MCF-7(0-800 mm³), MDA-MB-231(0-500 mm³), BT-474 (0-800 mm³) were collected and analyzed. For the breast tumors the groups were 100-250 mm³, 300-450 mm³, and 500-800 mm³. We collected non-localized Lac signal from 1D and 2D

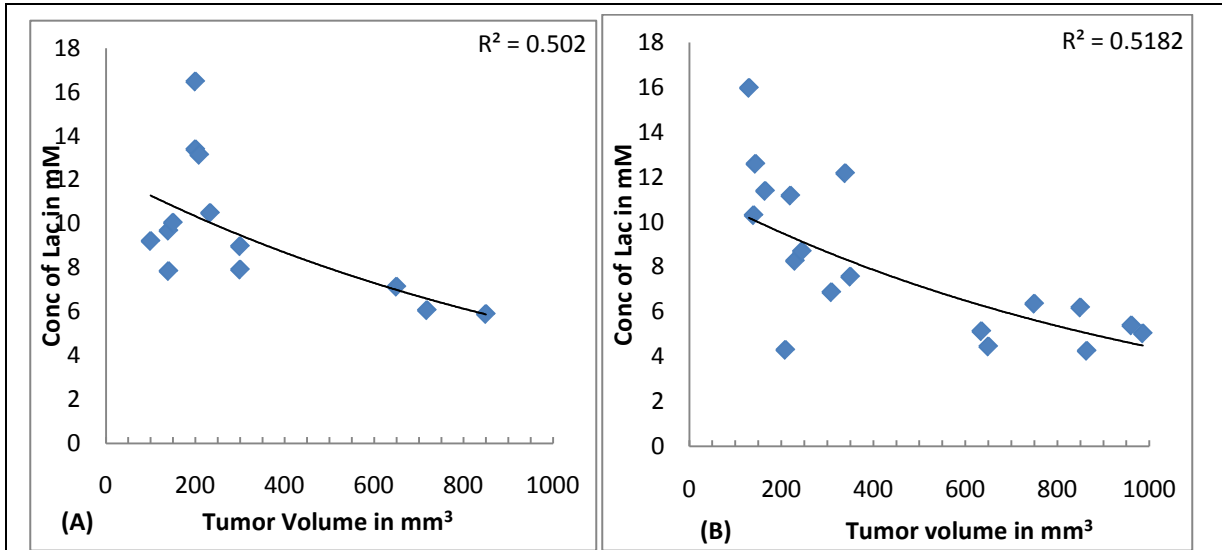


Figure 3: Plot of Lac concentration from whole 5mm slice as a function of tumor volume (A) BT-474, and (B) MCF-7. In both the tumor models the Lac concentration is high in the initial stage and decreases as the tumor volume increases. Histology is pending.

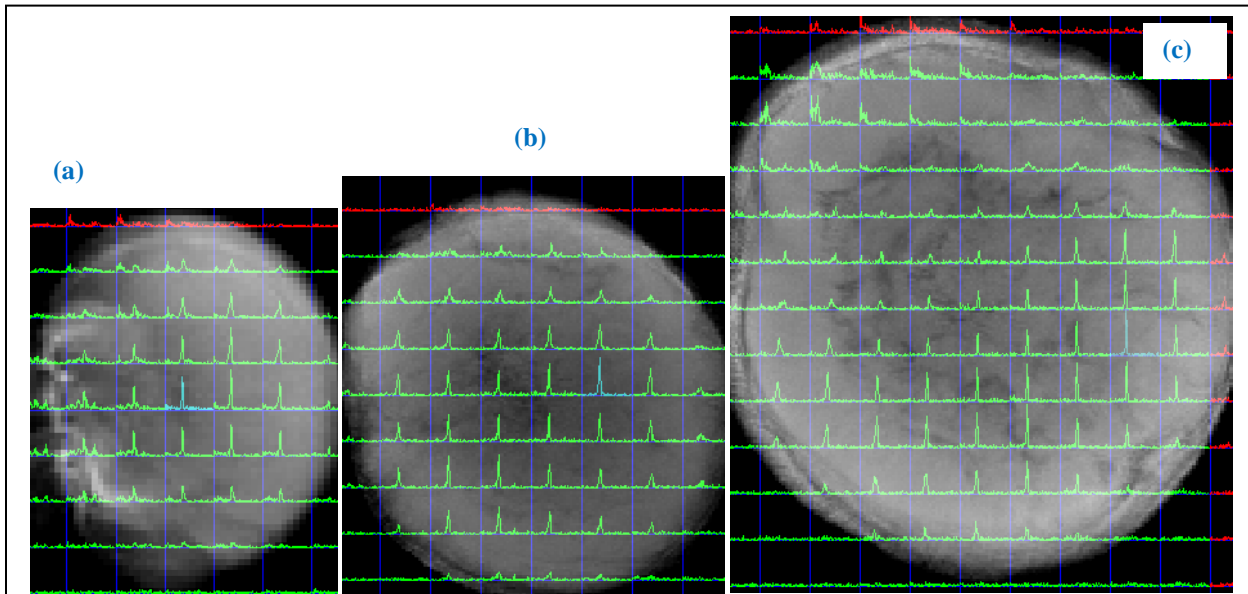
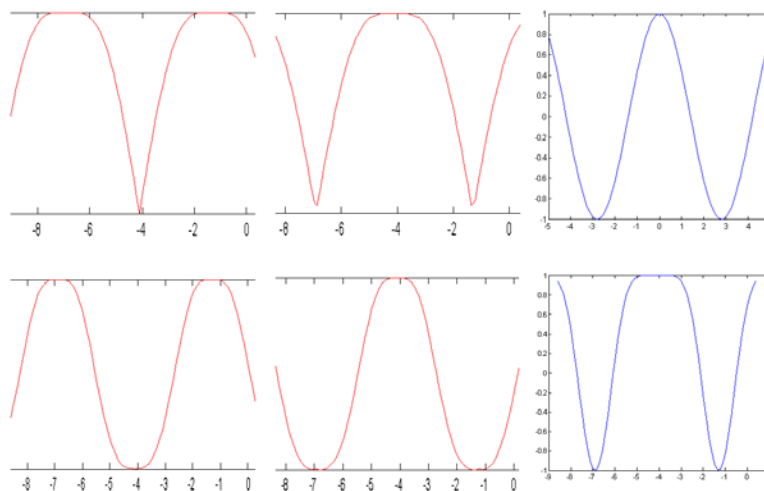


Figure 4: *In vivo* 2D Lactate CSI from 5 mm center sagittal slice across MCF-7 tumor with in plane resolution of 1.25x1.25 mm² at 4.7T using FOV=20mm with increasing tumor volume (a) 150mm³, (b) 330mm³ and (c) 750mm³; TR=2s; Number of scans=8 and acquisition time=76 minutes. Histology is pending.

chemical shift imaging data. We calculated concentrations from 1D signal using external reference phantom of 30mM lactate. 1D Lac spectra for MCF-7 with increasing tumor volumes are shown in Figure 1. Typically, in MCF-7 tumors, maximum concentration of Lac signal from a 5mm 1D slice selection within the volume of selection was found to be in the range of 10-15 mM (**Figure 3**). SKBR3 tumors were able to grow only up to less than 100 mm³ and got necrotic. Although we could detect Lac from whole tumors, no detectable signal in 2D CSI data was observed. Figure 4 demonstrates examples of the lactate spectra and the corresponding T2-weighted images obtained in the MCF-7 at three different sizes. We are in the process of calculating voxel-by-voxel lactate concentrations which may explain tumor heterogeneity.

Theoretical simulations: Performance of the selective pulses were evaluated using computer simulations of these pulse blocks using AX spin system representing lactate (A=CH₃; X=CH). Numerical simulations were generated using spin density matrix calculations. Selective excitation profile of binomial spectral-selective pulse is shown for CH₃ (-2.8 ppm) without disturbing CH (0 ppm) resonance (**Figure 5**) for 1-1 pulses (top) and 1331 (bottom). As seen from figures (Figure 5-bottom), on-resonant or off-resonant excitation regions are broader compared to pulses shown on top which facilitates better lipid suppression. The numerical simulations were



carried out using MATLAB signal processing package.

Figure 5: Theoretical excitation of off resonant, on resonant and off resonant inversion were represented as for SS-SelMQC-11 (**top**) pulse sequence and SS-SelMQC-1331 pulse sequence

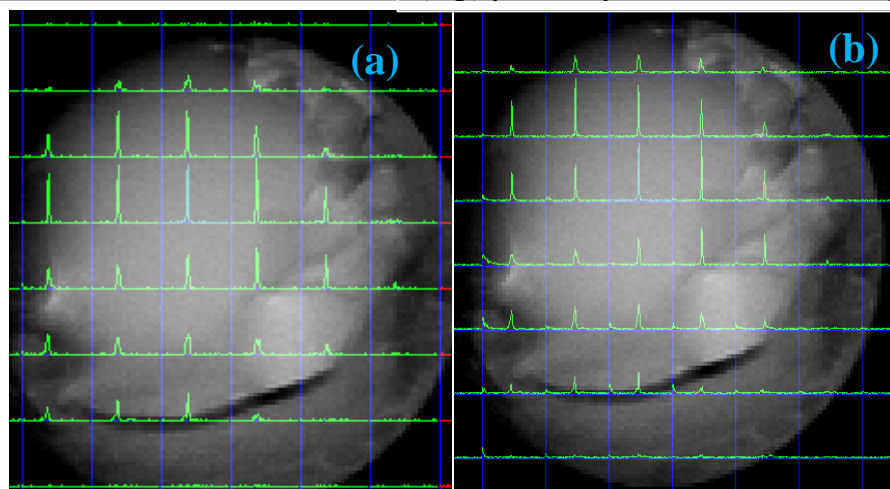


Figure 6: 2D CSI of 30mMol Lactate/lipid (a)SS-SelMQC-11 (b) SS-SelMQC-1331 sequence for in plane resolution of 1.25x1.25 mm² at 4.7T using FOV=20mm slice thickness is 5mm, TR=2s Number of scans=8 and acquisition time= 76 minutes. Better lipid suppression was observed.

Experimental Lac detection efficiency is compared using different methods (**Figure 6**). In comparison of frequency selective excitation of binomial pulses using 11 and 1331 in SS-SelMQC (1), spectral selection in the 1331 composite is broad, excites and refocuses better than 11 pulse (Manuscript under preparation).

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

1. Developed T_1 - and T_2 -versions of SS-SelMQC to measure T_1 and T_2 relaxation measurements for lactate quantification and tested with invivo tumors.
2. Developed SS-SelMQC with 1331 binomial pulses for better lipid suppression.
3. Implementation of quantitation methods

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

1. S. Annarao, K. Thomas, N. Pillarsetty, J. Koutcher, and S. Thakur, “In vivo lactate T_1 and T_2 relaxation measurements in ER-positive breast tumors using SS-SelMQC editing sequence”, *Proc. Intl. Soc. Mag. Reson. Med.* 19, 6210 (2011).

CONCLUSION:

We have made substantial progress with the evaluation of sequences as well as Lac detection in phantoms and *in vivo* tumors. As we have already collected the control data before treatment, We are very positive that within few months we will be able to collect the data of mice breast tumors following treatment using Lac concentration as a marker.

References

1. Thakur SB, Yaligar J, Koutcher JA. In vivo lactate signal enhancement using binomial spectral-selective pulses in selective MQ coherence (SS_SelMQC) spectroscopy. Magn Reson Med 2009 Sept; 62(3):591-598.
2. He Q, Shungu DC, van Zijl PC, Bhujwala ZM, Glickson JD. Single-scan in vivo lactate editing with complete lipid and water suppression by selective multiple-quantum-coherence transfer (Sel-MQC) with application to tumors. J Magn Reson B 1995 Mar; 106(3):203-11.
3. In vivo tumor lactate relaxation measurements by selective multiple-quantum-coherence (Sel-MQC) transfer Muruganandham M, Koutcher JA, Pizzorno G, He Q. Magn Reson Med 2004 Oct; 52(4): 902-906.
4. Accurate TI Determination from Inversion Recovery Images: Application to Human Brain at 4 Tesla Kim SG, Hu X, Ugurbil K Magn Reson Med 1994 Apr; 31(4): 445-449.

Appendices : Follows

S. Annarao¹, K. Thomas², N. Pillarsetty³, J. Koutcher^{3,4}, and S. Thakur^{3,5}¹Medical Physics, Memorial Sloan Kettering Cancer Center, New York, NY, United States, ²Molecular Pharmacology & Chemistry, Memorial Sloan Kettering Cancer Center, ³Radiology, Memorial Sloan Kettering Cancer Center, ⁴Medical Physics, Memorial Sloan Kettering Cancer Center, ⁵Memorial Sloan Kettering Cancer Center**Introduction**

Multiple quantum (MQ) editing techniques have been developed for lactate (Lac) detection with complete suppression of water and lipid (Lip) resonances in a single scan (1-3). In malignant tumors, due to elevated glycolysis, Lac may likely to be a marker for tumor diagnosis. A recent study (3) also reported that different tumor models exhibit varying average Lac concentrations at different tumor volumes with low Lac levels at smaller tumor volumes. In our previous work (SS-SelMQC) (1), we have reported 2-3 fold increase in the Lac signal to noise than SelMQC using in vivo rat prostate tumors. This method has high potential for studying breast tumors implanted on mice at smaller tumor volumes. Absolute quantification of Lac requires correction factors due to J-coupling evolution, molecular diffusion, T₁ and T₂ relaxation factors. Though we can calculate the effects of J-couplings and molecular diffusion effects, one needs to measure T₁ and T₂ for absolute quantification. Hence we report a modified T₁ and T₂ variants of SS-SelMQC sequence (1) to measure Lac T₁ and T₂ values with increased signal-to-noise as seen in the original sequence (Fig.1)(1). We measured T₁ and T₂ accurately using different phantom conditions as well as *in vivo* ER-positive breast tumors.

Materials and methods

Animals: MCF-7 and BT-474 cells were prepared with Matrigel. 4 to 6 weeks old Balb/c female nude mice were used and two days before the cell inoculation, estrogen pellet was induced in the mice body. 5×10^6 cells were inoculated on the mammary fat pad of the mice and tumor growth was started after one week of cell inoculation and tumor growth was monitored every week (Fig.2). The tumor volume was calculated by measuring the length (l) breadth (b) and height (h) of the tumor using the formula $\pi(l*b*h)/6$. Animal

studies were conducted in compliance with protocols approved by our institutional committee.

MR experiments: All experiments were performed on a 4.7 T Bruker Biospin spectrometer (40 cm horizontal bore). Studies were verified using Lac phantoms and demonstrated using a MCF-7 and BT-474 breast cancer tumor, subcutaneously implanted on mammary fat pad region of nude mice. The mice were anesthetized using a mixture of isoflurane and air (20% O₂) and placed in the animal holder. The tumor was placed inside a 2 turn home built 15 mm diameter tuned coil. MR spectroscopy acquisition parameters were same as our previous study (1,4) included a 45° and 90° pulse flip angle three-lobe sinc shaped pulses with 200μs and 400μs pulse duration, a pulse repetition of 3 s and spectral width of 12.5 ppm. Transmitter is set at CH frequency and all other experimental parameters are chosen from ref (1). The ZQ → DQ coherence transfer pathway is selected with the G_{sel} gradients in a ratio of 0:-1:2; Non-localized spectra were obtained with 16 transients for T₁ and 32 transients for T₂ measurements. In the T₁-SS-SelMQC, T₁ measurement of Lac was performed with insertion of inversion 'mao4' shaped pulse with 2000 μs pulse width, similar to (4) and varied the inversion time before applying the SS-SelMQC. In T₂-SS-SelMQC, Lac T₂ relaxation was measured by incorporating CH₃ selective 15ms single lobe 'sinc' pulse, during the MQ-preparation period of SS-SelMQC. This allows inserting a variable delay time TE' to measure Lac T₂ decay. The T₁ measurement was done by subtraction method (5).

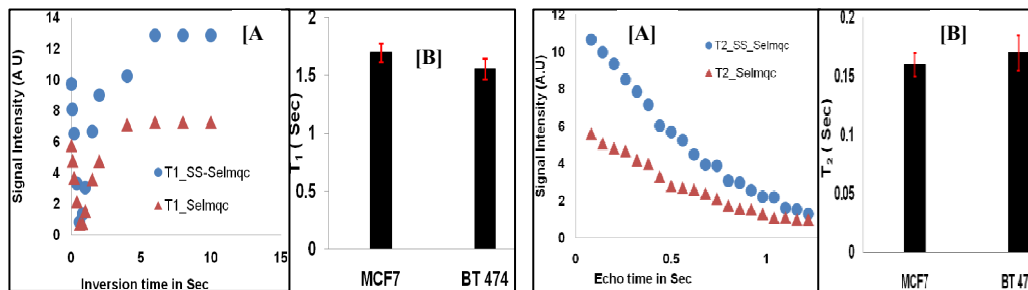


Fig 3:[A] The Lac signal recovery with variable recovery delay (0.1 to 1s) with 0.2 s increments and (1s to 10s) in steps of 2s. The signal intensity in T₁-SS-SelMQC (●) and T₁-SelMQC(Δ). Signal to noise increase observed in T₁-variant of SS-SelMQC [B]: The Lac T₁-values in MCF-7 and BT-474 tumors using SS-SelMQC variant.

Fig 4:[A] The Lac signal decay with $2*TE'$ (0.01 to 1s) with 0.02 s increments. The signal intensity in T₂-SS-SelMQC (●) and T₂-SelMQC(Δ). Signal to noise increase observed in T₂-variant of SS-SelMQC [B]: The Lac T₂-values in MCF-7 and BT-474 tumors using SS-SelMQC variant.

done using MCF-7 and BT-474 breast tumors in mice (N=3 in each group). The experimental recovery of Lac signal using T₁ variants of SS-SelMQC and SelMQC is shown in (Fig.3A). The T₁ relaxation times of Lac in MCF-7 and BT-474 breast tumors were found to be 1.7 ± 0.16 s and 1.56 ± 0.17 s respectively (Fig.3B). Similarly the T₂ measurements were done using T₂-variant of SS-SelMQC and compared with T₂-SelMQC (Fig.4). The exponential decay of Lac signal using T₂ variants of SS-SelMQC and Sel-MQC (Fig 4A). The increased signal enhancement was observed in T₂-SS-SelMQC, which facilitates to measure accurate T₂ of Lac signal in tumors with special advantage for tumors with low Lac levels. The T₂ relaxation times of Lac in MCF-7 and BT-474 tumors were found to be 0.16 ± 0.02 s and 0.17 ± 0.03 s respectively. Using phantoms of 5mM concentration, we observed marginal difference in T₁ value of Lac measured using both T₁-variants. But in T₂ measurements, increased signal to noise in T₂ of SS-SelMQC has a advantage of measuring the Lac peak areas more accurately than T₂-SelMQC due to shorter total echo time duration.

Table 1. T₁ and T₂ measurements of Lac solutions with Lip and Gd-DTPA doping

Conclusion: Using T₁- and T₂-variants of SS-SelMQC sequence, measured T₁ and T₂ values facilitates the absolute quantification of Lac concentrations *in vivo*.

Acknowledgement: We want to thank Dr. Mihaela Lupu and Ms.Natalia Kruchevsky for their help. This work is supported by DOD BCRP award W81XWH-09-1-0042.

References: (1) Thakur SB, et al., Magn Reson Med 2009; 62: 591-598. (2) He Q, et al., J. Magn. Reson. B 1995; 106: 203-211. (3) Yaligar J, et al., PISMRM 2008: 369. (4) Muruganandham M, et al., Magn Reson Med 2004; 52: 902-906. (5) Kim S, et al., Magn Reson Med 1994; 31: 445-449.

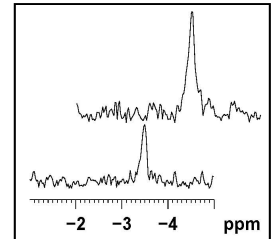


Fig 1. Lactate spectra of SS-SelMQC(top) and SelMQC(bottom); Signal enhancement > 2 times in SS-SelMQC was observed. MCF-7 with tumor volume 230mm³

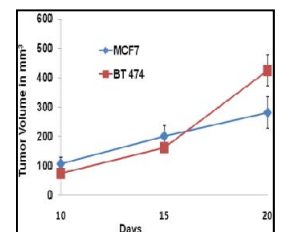


Fig 2: Plot of tumor volume of MCF-7 and BT-474 induced breast tumors in mice.

Results and Discussion:

The *in-vitro* T₁ and T₂ of Lac CH₃ signal was also measured in phantom using different Lac concentrations (5, 15, 30 mM) and in different chemical environment (in absence of Lip, in presence of Lip and in presence of contrast agents) all the solutions were prepared in physiological saline. The respective T₁ and T₂ of Lac -CH₃ signal is presented in Table 1.

In vivo T₁ and T₂ measurements were

Lac Concentration (mM)	T ₁ (sec)	T ₂ (sec)
5	1.34±0.05	0.60±0.02
15	1.21±0.02	0.51±0.03
30	1.13±0.03	0.50±0.03
15 with lipid	1.2±0.01	0.55±0.02
30 with lipid	1.10±0.08	0.54±0.03
15 with 25 μMol Gd-DTPA	0.66±0.01	0.35±0.03
30 with 25 μMol Gd-DTPA	0.74±0.02	0.36±0.01
30 with 50 μMol Gd-DTPA	0.67±0.01	0.31±0.01